Experiment 7

Date : 28-1-23

RBC COUNT

AIM

To find out the number of red cells in one cubic milli meter of blood.

PRINCIPLE

The number of RBCs in a known volume of diluted blood is counted and the number of cells in one mm^3 of undiluted blood is calculated from this.

APPARATUS

Hemocytometer, RBC diluting fluid, compound microscope, sterile lancet, watch glass, cotton, rectified spirit.

Improved Neubauer double counting chamber

This is a thick rectangular glass slide with a polished transverse bar in the center, separated from the rest of the slide by two parallel grooves on the either side. The polished bar is divided into two equal platforms by a groove in the middle. The surface of the platforms is 1/10mm below the surface of the rest of the slide. So, If a cover glass is placed over the surface of the counting chamber, the under surface of the cover glass remains 1/10mm above the polished surface of each platform. The counting area is in the form of central ruled area on the polished surface of each platform. It is a square of 3mm side, divided into 9 equal squares 1mm side. Of these, the four corner squares are used for WBC counting. Each WBC square of 1mm side is again divided in to sixteen smaller squares each of 1/4mm side. The central 1mm square is divided in to 25 equal smaller squares of 1/5mm side, of which the 4 corner ones and the central one are used for R.B.C counting. Each of these squares is subdivided into 16 smallest squares each of 1/20mm side.

R.B.C. Pipette

This is a bulb pipette having a long stem with a capillary bore and a pointed tip. The bulb contains a red bead inside. To the small narrow portion above the bulb is connected a small rubber tube provided with a mouthpiece for sucking blood and fluid into the pipette. The pipette has three markings on it, 0.5 mark in the middle of the stem, 1 mark at the junction between stem and bulb, and 101 marks above the bulb. The total volume of the pipette is 101 parts, of which one part is in the stem and 100 parts in the bulb.

Other uses of R.B.C. pipette

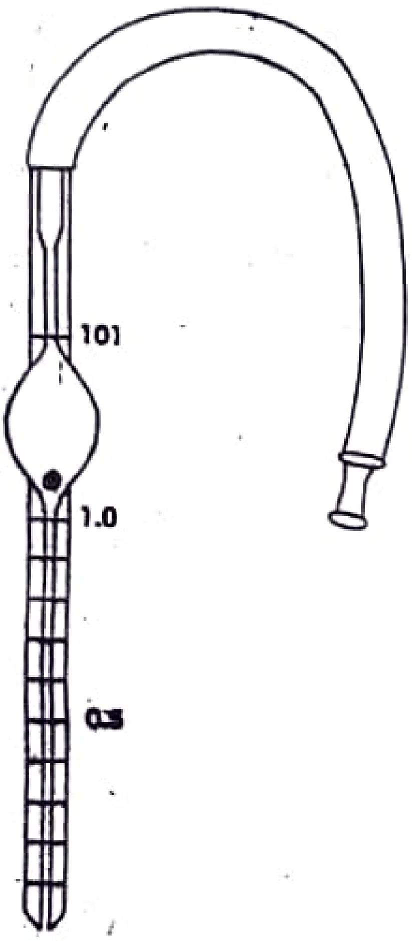
1. WBC count in leukemia
2. Platelet counting
3. Sperm Count

Uses of the bead in the bulb

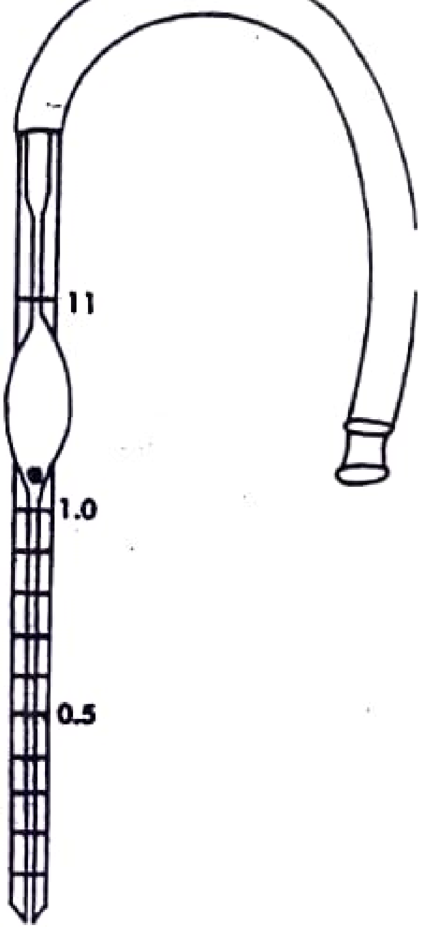
1. For proper mixing
2. To know whether the pipette is dry
3. To identify the pipette

R.B.C. diluting fluid

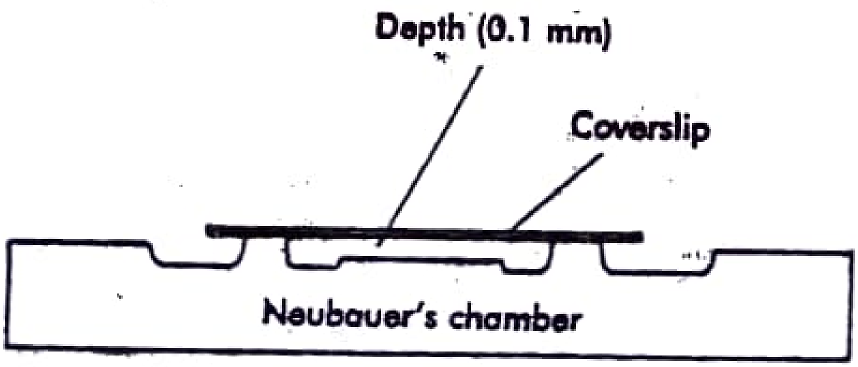
Hayem's fluid is the commonly used diluting fluid.



RBC pipette



WBC pipette



Side view of Neubauer's chamber showing the space between the under surface of the coverslip and the surface of the platform (0.1 mm deep)

Composition:

Sodium Chloride	0.5gm
Sodium Sulphate	2.5gm
Mercuric per chloride	0.25gm
Distilled water	100cc

Sodium Chloride and Sodium Sulphate together keeps the isotonicity of fluid. Sodium Sulphate also prevents clumping of red cells. Mercuric per chloride fixes the cells and acts as preservative. The fluid has to be renewed rather frequently.

Other diluting fluids:

Gower's fluid
Toison's fluid
Dacies Formol Citrate solution (best diluting fluid)

PROCEDURE

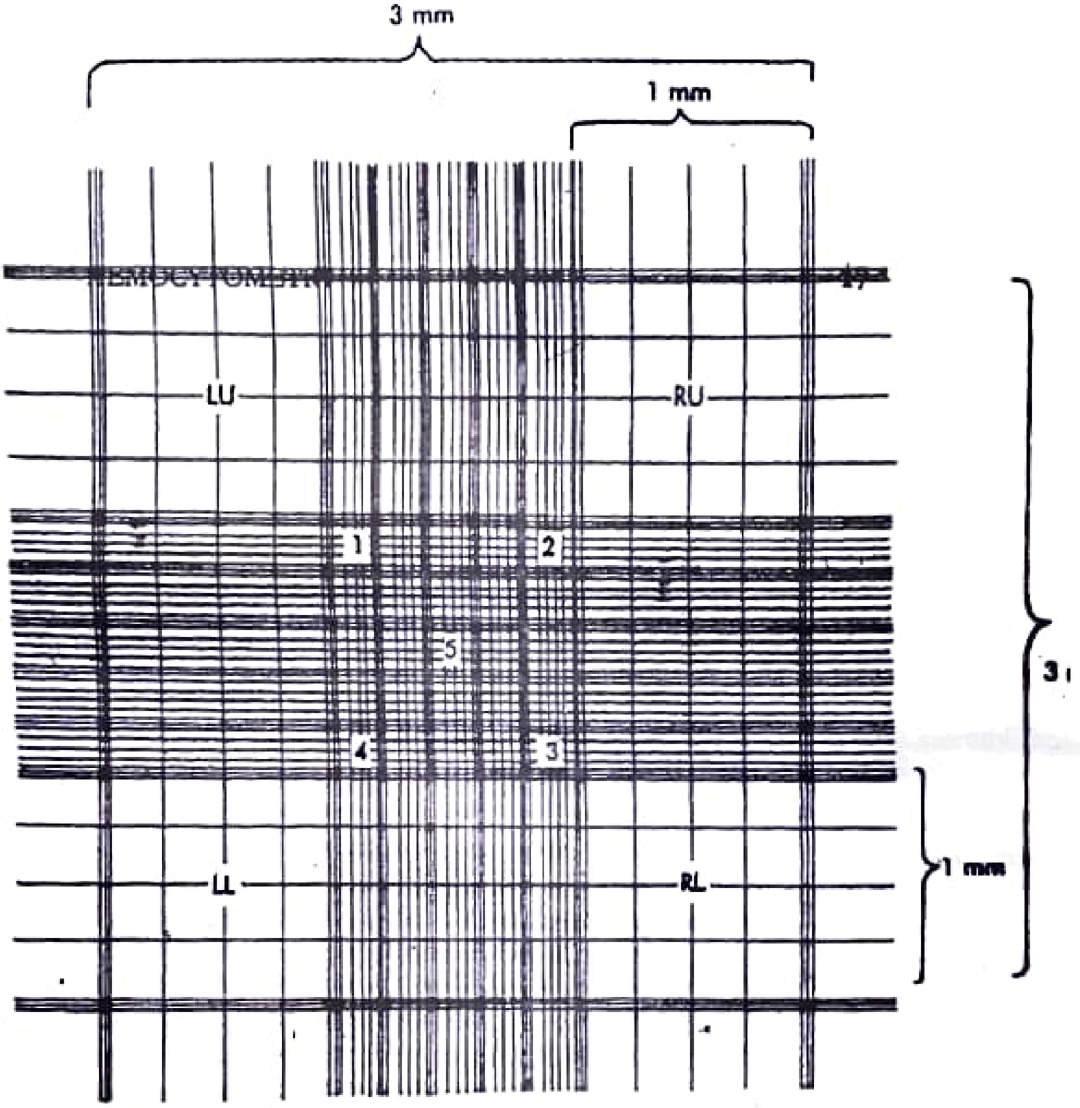
Clean and dry the counting chamber and put on the special cover slip provided. Focus under the high-power objective and identify the RBC counting area, clean the RBC pipette first with distilled water, then with absolute alcohol and finally with ether and keep it dry. Take a small quantity of diluting fluid in a watch glass and keep aside. Clean the fingertip using rectified spirit and make a deep prick with a sterile lancet so that blood comes out freely without squeezing. Wipe off the first drop, which may contain tissue fluid also. Allow a good-sized blood drop of form. Keeping the pointed top of the pipette touching the drop, suck in blood up to the 0.5 mark carefully, without any air bubble. Excess blood at the tip of the pipette is removed using a blotting paper or a piece of cotton. Immediately, diluting fluid from the watch glass is sucked in up to the 101 mark, taking care not to allow any air bubble to enter.

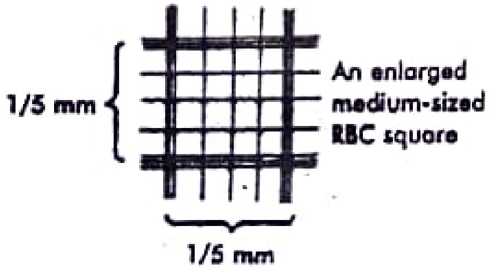
Thoroughly mix the blood and diluting fluid in the pipette by gently rolling the pipette, held horizontally between the palms and keep aside. Mixing takes place only in the bulb of the pipette. Now take out the counting chamber for charging. Discard first few drops from the pipette, as the stem contains only diluting fluid. Bring one small drop of diluted blood at the tip of the pipette, to the edge of the cover slip on the counting chamber at an angle much less than 45. The fluid enters by capillary action under the cover slip and fills the counting chamber. Both areas are filled. Focus the RBC counting area under high power. Keep the counting chamber undisturbed for about 3 minutes for the cells to settle down in the counting area. At least 5 squares, each having 16 smallest squares (preferable 4 corners and 1 central) should be counted to obtain a satisfactory average and a better dispersal value. While counting each small square, cells touching the top and left margin of each square should be omitted and cells touching bottom and right margin of each square should be counted. Draw a chart of the counting squares in the record and enter the number of cells in each square as and when counted.

Precautions:

1. Counting chamber and pipette should be clean and dry
2. Fingertip and pricking lancet should be sterile
3. Blood should freely come out without squeezing
4. Be careful to prevent clotting of blood inside the pipette
5. While filling the pipette and charging the counting chamber, no air bubbles should enter.
6. While charging the counting chamber, over filling and spilling should be avoided.
7. Cells should settle down and should be more or less evenly distributed before counting.
8. Take care to see that no cell is counted more than once and all cells in the area are counted.

HEMOCYTOMETRY





Improved Neubauer's Chamber
LU : left upper; right upper
LL : left lower; RL : right lower

CALCULATION

Side of 1 square = 1/20mm

Area of 1 square = 1/400 mm^2

Depth of the fluid in counting chamber is 1/10mm

Volume of diluted blood in 1 square = $1/400 \times 1/10 = \frac{1}{4000}$ mm^3

Volume of diluted blood in 80 squares = $80 \times \frac{1}{4000}$ mm^3

Number of cells in 80/4000 mm^3 diluted blood = N

Number of cells in 1mm^3 of diluted blood = $\frac{N}{80 \times 1/4000} = \frac{N \times 4000}{80} = N \times 50$

Dilution factor:

Total diluted volume in bulb of the pipette is 100 parts. Out of which 0.5 part is blood. So, dilution is 0.5 in 100 i.e. 1 in 200. Dilution factor is 200.

So, the number of cells in 1mm^3 of undiluted blood = $N \times 50 \times 200 = N \times 10000$ (Expressed in millions /mm^3)

Discussion

Normal RBC count

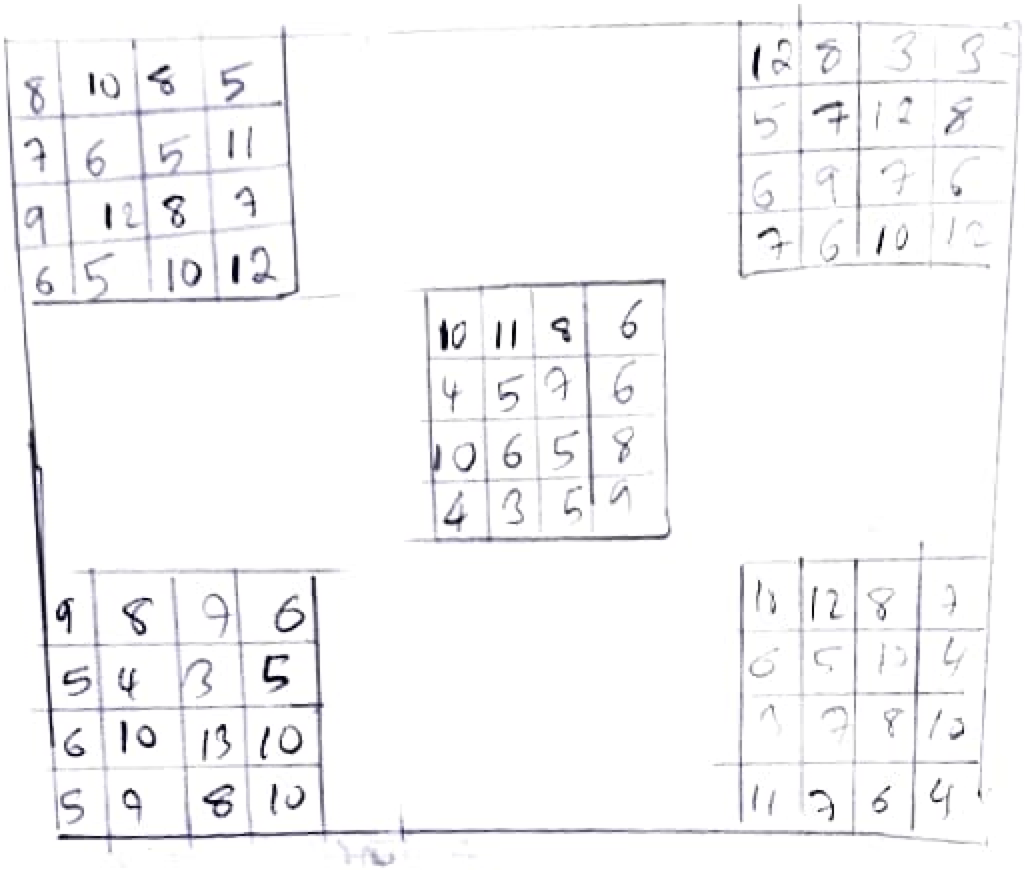
Adult male	5 – 5.5 millions/mm^3
Adult female	4.5 – 5 millions / mm^3

Variation in count

Increase in count	Polycythemia (Erythrocytosis)
Decrease in count	Anemia (Erythropenia)

Physiological variations

1.	Diurnal	- less during night, minimum in early morning, gradual increase during day.
2.	Age	- in new born high count
3.	Sex	- count in females lower than in males
4.	High altitudes	- count higher due to hypoxia
5.	Muscular exercise	- count increases
6.	Pregnancy	- count decreases due to hemodilution



8	10	8	5
7	6	5	11
9	12	8	7
6	5	10	12

12	8	3	3
5	7	12	8
6	9	7	6
7	6	10	12

10	11	8	6
4	5	7	6
10	6	5	8
4	3	5	9

9	8	9	6
5	4	3	5
6	10	13	10
5	9	8	10

10	12	8	7
6	5	10	4
9	7	8	10
11	7	6	4

N = 590

Calculation

Side of 1 square = $\frac{1}{20}$ m

Dilution factor = 200

Area of 1 square = $\frac{1}{400}$ mm²

Depth of fluid = $\frac{1}{10}$ mm

Volume of diluted blood in 1 square =

$$\frac{1}{400} \times \frac{1}{10} = \frac{1}{4000} \text{ mm}^3$$

Volume of diluted blood in 80 squares =

$$80 \times \frac{1}{4000} \text{ mm}^3$$

Number of cell in $\frac{80}{4000}$ mm³ dilute blood (N) = 567

No of cells in 1 mm³ by undiluted blood = N × 50 × 200
= N × 10000
= 567000 cells/mm³

Pathological increase in count

1. Lung diseases like emphysema, pulmonary tuberculosis
2. Congenital cyanotic heart diseases
3. Carbon monoxide poisoning
4. Primary polycythemia (Polycythemia rubra Vera)
5. Hemoconcentration

Secondary polycythemia is due to hypoxia resulting from any cause, physiological or pathological.

Pathological decrease – Anemia

1. Increased destruction of RBC:Hemolytic anemias
2. Decreased production of RBC: Aplastic anemia, nutritional anemias, anemia of chronic renal failure
3. Following hemorrhage (acute / chronic)

Anemia is defined as the quantitative or qualitative decrease in RBC count or hemoglobin concentration below the lower limit of normal range for the age and sex of an individual.

Result :

Number of cells in 1mm³ of undiluted blood

=> 5.67 million cells/mm³.

Questions-

1. Diagrammatically represent various stages of Erythropoiesis.
2. Differentiate between primary and secondary polycythemia.
3. List the factors affecting erythropoiesis.
4. Why should you discard two drops from pipette before charging the chamber?
5. What are the sources of error in this experiment?
6. Why is RBC count more in males?

(1) STAGES OF ERYTHROPOIESIS

PLURIPOTENT HEMOPOIETIC STEM CELL

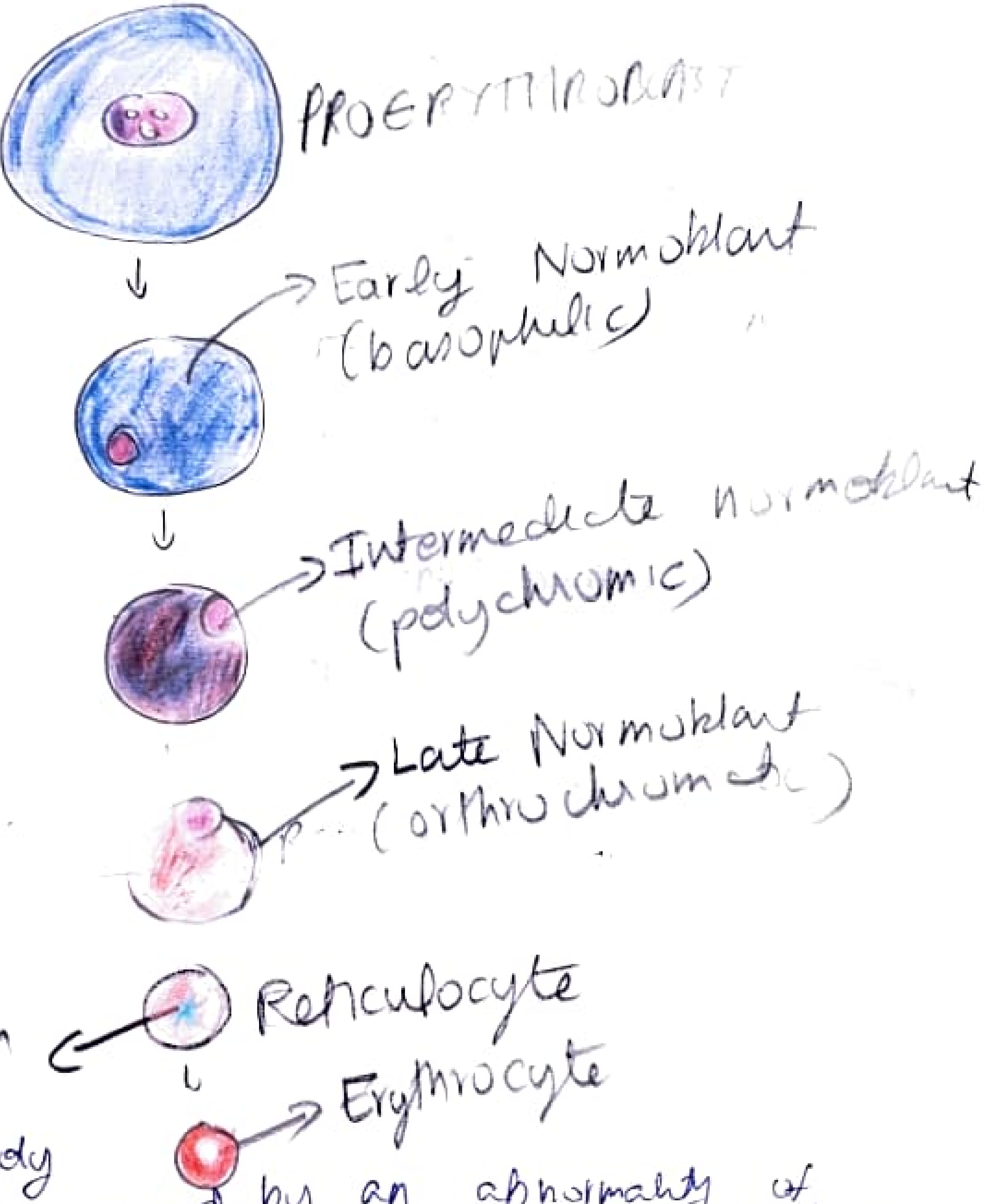
↓

BFU-E

↓

HEMOCYTOBLAST [CFU-E]

↓


PROERYTHROBLAST
Early Normoblast (basophilic)
Intermediate normoblast (polychromic)
Late Normoblast (orthochromatic)
Reticulocyte
Reticulum
Erythrocyte

2. Primary polycythemia is caused by an abnormality of the cells in the bone marrow that form red blood cells. Secondary polycythemia is caused by a disorder origination outside of the bone marrow that causes overstimulation

of the normal bone marrow leading to an overproduction of red blood cells.

3. (i) General factors
   - Erythropoietin [Hyposia)
   - Thyroxine [Growth inducers).
   - Hemopoietic growth factors
   - Vitamin

(ii) Maturation factor
   - Vitamin B12 (Cyanocobalamin)
   - Intrinsic factor of Castle
   - Folic acid.

(iii) Factors necessary for hemoglobin formation.

(4) The first two drops coming from the capillary stem should be discarded because these drops are diluting fluid which is all free. The chamber shoul be charged at one go and not i points. For this one needs a drops of proper size.

(5) *Pipetting incorrect volume of sample
* Air bubble during pipetting.
* less hemoglobin
* Human error [mixing, handling, dilution, miscalculation, procedure]
* Instrumentation and material variation
[grids, depth, coverslips, types of butter pipette

6) In females estrogen hormone is produced which has an inhibitory effect on erythropoietin; whereas testosterone produced in male favours erythropoietin production thus increasing RBC count in males

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Experiment 8 Date: 16-01-23

BLOOD INDICES

The blood indices of clinical value are:

(1) Mean corpuscular volume (MCV)

(2) Mean corpuscular Haemoglobin (MCH)

(3) Mean corpuscular Haemoglobin concentration (MCHC)

Blood indices help in the classification of different types of anaemia. They are calculated from the values of PCV, RBC count and haemoglobin content of blood.

Mean Corpuscular volume (MCV)

Average volume of a single red cell, expressed in cubic microns.

$$\text{MCV} = \frac{\text{PCV} \times 10}{\text{RBC count in millions / cu.mm}}$$

Normal value is 86+/- 10 cubic microns

An RBC with normal MCV is a normocyte, that with increased MCV is a macrocyte, eg:- Megaloblastic anemia and that with a decreased MCV is a microcyte. eg. Iron deficiency Anemia.

Mean Corpuscular Haemoglobin (MCH)

Average amount of haemoglobin contained in a single red cell and is expressed in picograms.

$$\text{MCH} = \frac{\text{Hb content in gm/dl} \times 100}{\text{PCV}}$$

Normal value is 34 +/- 2%

MCHC is decreased in iron deficiency anaemia. Here MCV and MCH are decreased, but MCH is decreased out of proportion to the reduction in cell volume. So MCHC is decreased and the cell is not fully saturated with hemoglobin. In pernicious anemia, MCHC remains normal, since MCV and MCH increase proportionately. The cell is fully saturated with hemoglobin. Under no circumstances can the value of MCHC can exceed the upper limit of normal, because the RBC cannot be supersaturated with hemoglobin. MCHC is more reliable than MCH, because PCV and blood hemoglobin concentration are more accurate than RBC count.

Colour Index (CI): is the ratio of blood Hb concentration expressed as a percentage of normal to RBC count, expressed as a percentage of normal. Being a ratio, CI has no units. CI reflects the amount of Hb in each RBC in relation to amount of Hb in a normal RBC. For calculating CI, normal blood Hb concentration is taken as 14.5gm/dl and normal RBC count as 5millions/cumm.

$$CI = \frac{\text{(observed Hb conc x 100) / normal Hb conc}}{\text{(observed RBC count x 100) / normal RBC count}}$$

= (observed Hb conc x normal RBC count) / (normal Hb conc x observed RBC count)

= (observed Hb conc x 5million/cumm)/(observed RBC count x 14.5gm/dl)

Normal CI = 0.8 – 1.2, average 1. Increased in macrocytic anemias. Decreased in iron deficiency anemia. But Hb conc and RBC count may decrease proportionately to give normal CI in anemia. So, CI is not of much clinical significance.

Other blood indices: Reb cell distribution width (RDW), hemoglobin distribution width (HDW), cellular hemoglobin concentration direct measurement (CHCM), and mean platelet volume (MPV) are obtained using automated cell counters.

Uses of blood indices: To know the morphological type of anemia.

Question-

1. What is macrocytosis and microcytosis? What are their causes?
2. Calculate the blood indices from the Hb, RBC Count and PCV Values

Macrocytosis is a term used to describe red blood cells that are larger than normal. It is also known as megalocytosis or macrocythemia. On this condition, the RBC mean volume (MCV) will be higher than ~~e~~ suspected value cause.

- Vitamin B12 deficieny.
- Folate deficiency
- Liver disease.
- Alcoholism.
- hypothyroidism.

Microcytosis is a descriptive forms for red blood size smaller than normal range. It is also known as microcythemia. MCV lower than expected value.

Cause:- Iron deficiency anemia.
- thalassemia.
- mutations

2. I $$MCV = \frac{\text{Haematocrit \% × 10}}{\text{RBC count (millions/mm}^3\text{)}}$$

II $$MCH = \frac{\text{Hb (g/dL) × 10}}{\text{RBC count (millions/mm}^3\text{)}}$$

III $$MCHC = \frac{\text{Hb (g/dL) × 100}}{\text{Haematocrit \%}}$$

eg:- 1) Haematocrit % = 45%, RBC count = 5 million/mm3, Hb = 15g%.

Calculate MCV

$MCV = \frac{45 \times 10}{105} = 90fL$

(2) Hb = 15g%, RBC count = 5 million/mm³,

PCV = 45%.

Calculate MCH

$MCH = \frac{15 \times 10}{5} = 30pg$.

(3) PCV = 45%, Hb = 15g/dL.

Calculate MCHC.

$MCHC = \frac{15 \times 100}{45} = 33.33\%$

Experiment 9

Date :

TOTAL LEUCOCYTE COUNT

AIM

To determine the total leucocyte count of a sample of blood.

PRINCIPLE

The number of WBCs in a known volume of diluted blood is counted and from this the number of WBCs in 1 cube millimeter of undiluted blood is calculated.

APPARATUS

Hemocytometer with WBC pipette, diluting fluid, compound microscope, sterile lancet, watch glass, rectified spirit, cotton.

Diluting fluid

Ideal diluting fluid should dilute the blood, destroy RBCs but not WBCs and stain the nucleus of WBCs.

Diluting fluid used is Turk's fluid. Composition is

Gentian violet	- 0.25gm
Glacial acetic acid	- 2cc
Distilled water up to	- 100ml

Gentian violet stains the nucleus of WBCs and acetic acid destroys the RBCs by hemolysis.

WBC Pipette

This a bulb pipette having a long stem with a capillary bore and a pointed tip. The bulb contains a white bead inside. To the small narrow portion above the bulb is connected a small rubber tube, provided with a mouth piece for sucking blood and fluid into the pipette. The pipette has three markings on it, 0.5 mark in the middle of the stem, 1 mark at the junction between stem and bulb, and 11 mark above the bulb. The total volume of the pipette is 11 parts of which one part is in the stem and 10 parts in the bulb.

Other uses of W.B.C. pipette:

1. Cell count in C.S.F.
2. Absolute eosinophil count
3. R.B.C. count in severe anaemia

Procedure

Clean the counting chamber and the cover slip with water, dry it and mount it on the mechanical stage of microscope. Focus any one of the four corner squares under the low power. Take a small quantity of the diluting fluid in a watch glass. See that the pipette is clean and dry.

Clean the fingertip and allow it to dry. Using a sterile needle make a sufficiently deep prick, so that blood flows freely. Discard the first drop, which may contain tissue fluid. Suck in blood up to the 0.5 mark of WBC pipette followed by the diluting fluild up to the mark 11, from the watch glass. Allow the blood and diluting fluid to mix well by rolling the pipette horizontally in between the two palms.

3	5	9	5
6	4	3	2
1	2	5	7
9	5	4	2

2	4	4	5
4	3	5	3
3	3	4	2
2	1	2	2

1	2	1	1
2	8	3	6
2	1	2	3
3	3	4	3

2	2	1	5
6	2	8	1
4	4	1	1
2	1	3	2

Side = $\frac{1}{4}$ mm

Area of 1 square = $\frac{1}{16}$ mm^2

Depth of fluid film = $\frac{1}{10}$ mm

Volume of fluid in 1 square = $\frac{1}{16} \times \frac{1}{10} = \frac{1}{160}$ mm

No. of cells counted in 64 squares

No. of cells in $\frac{64}{160}$ mm 3647 x 168 x 2 160 80 40 20 x5 mm of diluted blood = N

No. of cells in 1mm^3 = $\frac{N}{2} \times 5$

Dilution factor = $\frac{1}{200}$

No. of cells in 1mm^3 of undiluted blood is

$= \frac{N}{2} \times 5 \times \frac{1}{200}$ 200 = N × 50 = 212 × 50 = 10,600 cells

The counting chamber is charged, taking all precautions, using the diluted blood from the pipette, touching the edge of the cover slip on the chamber with a small drop at the tip of the pipette. Keep the counting chamber undisturbed for 2-3 minutes on the stage so that the cell settles down on the ruled area. Then count the cells under low power. Draw a chart of the squares and enter the number of cells in each square while counting.

Precautions

1. Counting chamber, pipette, fingertip and lancet should be clean and dry.
2. No air bubble should be present in pipette or counting chamber.
3. There should not be any overflowing in the chamber.
4. The cells touching the right and upper border should be omitted. This is to prevent counting the same cells twice.
5. Blood should not be allowed to clot in the pipette.
6. Cells should not be moving, and should be more or less equally distributed.

Calculation

Side length of 1 square - ¼ mm

Area of 1 square - 1/16mm^2

Depth of fluid film - 1/10mm

So, volume of fluid in 1 square = 1/16 x 1/10 = 1/160mm^3

Let the number of cells counted in 64 squares be 'N'

No. of cells in 64/160mm of diluted blood = N

No. of cells in 1 cu.mm of diluted blood $= \frac{N}{64} \times 160$

Dilution = 1/20

No. of cells in 1 cu. mm of undiluted blood $= \frac{N}{64} \times 160 \times 20 = N \times 50$ cells / cu.mm

Discussion

Normal value: 4,000 – 11,000 cells / cubic millimeters of blood

Variations

Increased count is called leucocytosis

Decreased count is called leucopenia

Leucocytosis

Physiological

Pathological

Physiological

1. After exercise
2. During emotional outburst
3. After meals
4. Menstruation, pregnancy, Labor – return to normal after delivery
5. At birth. WBC count is more in children than in adults.
6. Diurnal variations: Lowest in morning, rises during midday to reach highest value in evening.

Pathological

1. Acute pyogenic bacterial infections. Eg. Pneumonia, abscess
2. Acute hemorrhage : starts within few hrs of hemorrhage
3. Trauma :mainly surgical operations, fractures, etc.
4. Burns
5. Collagen disorders : acute phases of rheumatoid arthritis
6. Leukemia

Leukemia

These are malignant diseases of unknown etiology characterized by uncontrolled abnormal and widespread proliferation of leucocyte precursors, which infiltrate the bone marrow and other body tissues and pill into circulating blood. This proliferation is usually accompanied by the appearance of immature leucocytes in peripheral blood, which are morphologically abnormal. Broadly classified into acute and chronic leukemias, based on course. Classified into myeloid (including monocytic leukemia also) and lymphatic leukemias. WBC count is very high in leukemias.

Leucopenia

Below 4000/cu.mm

1. A few bacterial infections eg. Typhoid, Paratyphoid and Brucellosis
2. Viral infectionseg. Infective hepatitis, Measles, Rubella, influenza, small pox
3. All types of overwhelming infections eg. Miliarytuberculosis, septicemia, fatal case of pneumonia.
4. Drug induced eg. Chloramphenicol (chloromycetin), oxyphenbutazone, idomethacin, anticancer drugs.

Result

Total WBC count of my own blood is 10,600 cells/mm^3 ✓

Questions-

1. What are the differences between leukocytosis and leukemia?
2. How can you differentiate WBC from other artifacts?
3. Differentiate between RBC and WBC pipette?

(1) Leukocytosis is just an increase in cell number of WBC, while this would happen in leukemia. Leukemia is malignant proliferation of WBC. It is a cancer

(2) It is differentiated from a blood smear obtained with a polychromatic stain which differentiate from other cells.

wbcs have halo around them

(3)

RBC pipette	WBC pipette
Red bead in the bulb.	white bead in the bulb.
Has 101 marks above the bulb.	Has 11 marks above the bulb.
Total volume of pipette is 101 parts. ↓ 1 part in stem + 100 parts in bulb.	Total volume of pipette is 11 parts ↓ 1 part in stem + 10 parts in bulb
Size of bulb is larger.	Size of bulb is smaller.
is smaller Size of lumen P or capillary bore lumen is narrow thus it is slow speed pipettes	Size of lumen is larger or capillary bore lumen is wider thus it is fast speed pipette.
Mouth piece red in colour.	Mouth piece white in colour

Date :

Experiment 10

PERIPHERAL BLOOD SMEAR

AIM

To prepare, stain and examine and ideal blood smear

APPARATUS

Clean, dry, grease, free glass slides with smooth edges, a drop bottle containing Leishman's stain, distilled water, ceder wood oil, microscope, rectified spirit, lancet, empty wash bottle.

Leishman's stain

It is a type of Romanowsky stain, which contains an acidic dye and a basic dye. The acidic dye stains the basic components of the cell and the basic dye stain the acidic components of the cell. The different cells are differentially stained. Leishman's stain contains eosin and methylene blue in acetone free methyl alcohol. Methyl alcohol acts as a fixative. (acetone if present, will destroy the cell membrane) Methylene blue, the basic dye and eosin, the acidic dye exists as thiazine eosinate, which dissociates into the component dyes, when diluted with distilled water. 0.15gm. Leishman's stain powder is dissolved in 100ml acetone free methyl alcohol, filtered and kept for 2-3 weeks.

Other Romanowsky stains include Wright's stain, Giemsa's stain, Jenner's stain, etc.

PROCEDURE

Preparation of peripheral smear:

Clean the slides using soap and water and dry using a fresh hand kerchief. Choose a slide with smooth edges as the spreader slide. Obtain a moderately sized drop of blood by finger puncture method and place it about 2cm from one end of the slide. Keep the slide horizontally on the table. Hold the spreader slide between the thumb and index finger. Place the narrow edge of the spreader slide in front of the blood drop at an angle of 45°. Bring it slowly backwards till it touches the blood drop. Blood spreads along the edge of the spreader by capillary action. When the blood spreads to about 2/3 of the width of the slide, the spreader is pushed forwards with uniform speed and pressure. Dry the smear in the air.

Staining the smear: The dried smear is placed horizontally on a staining rack. Pour stain drop by drop till it covers the smear. Wait for 1-2 minutes. Then dilute the stain using distilled water, double the amount of stain. A golden scum is formed over the diluted stain. Mix gently by blowing air. Keep it for 7-10 minutes, when the smear gets stained. Wash the smear in running tap water till the smear turns pink and the water flowing out is colorless. Take care to see that the tap water does not fall directly on the smear. Wipe the back of the slide and keep it in a vertical position to drain and dry. The film is first examined under the low power. Focus a well-stained area where the cells are uniformly distributed. Put one drop of cedar wood oil on the smear and examine under oil immersion objective. It is ideal to prepare and stain 2 or 3 peripheral blood smears simultaneously.

Criteria for a good smear

1. An ideally taken smear will be tongue shaped.
2. The smear should occupy only the middle two thirds of the slide
3. The smear should not be serrated or wavy and should not contain any holes.
4. It should neither be too thick nor too thin. Large drop, faster stroke and greater angle between slides make the smear thicker.
5. The stained smear should be pink in color and free from stain particles.
6. RBCs should be pink in color and WBCs should take up appropriate color.

Use of peripheral smear

1. To get an idea about the relative proportion of RBCs, WBCs and platelets.
2. To study the size, shape and distribution of RBCs.
3. To detect anemia. It also helps to differentiate the different types of anemia
4. To detect inclusion bodies and parasites (in RBCs). Eg.: Malarial parasites, microfilaria, toxoplasmosis, basophilic stippling in lead poisoning.
5. To have a rough idea about the number and distribution of WBCs.
6. To detect the presence of any abnormal or immature cells as in leukemia
7. To get a differential leucocyte count.
8. To have a rough estimate of the number of platelets.

OBSERVATION

In an ideally stained smear RBCs are the most numerous cells in a field. They are evenly distributed without touching each other. They are pink in color with periphery darker than the center. Cell size is about 7.5 microns in diameter.

The different types of leucocytes are not uniformly distributed in the film. They can be differentiated by the color taken up during staining. The granulocytes can be differentiated by the color and nature of the granules and shape of the nucleus.

Platelets are oval or round, purple stained particles seen in groups. They are small bodies (2-3 microns) showing a pale periphery and a deeply stained central part, which gives the appearance of pseudo nucleus.

Result

The RBC were pink in color with a zone of central pallor occupying $\frac{1}{3}$rd of size of RBC. Leukocytes could be differentiated, different types of WBCs could be seen

Questions

1. What are the qualities of a good smear?
2. Why is cedar wood oil used with oil immersion objective?

(1) Qualities of good smear:-

(i) Thick at ends and becomes progressively thinner with good separation of cells towards the opposite end.

(ii) The spreader should have smooth end to prevent the tail end of smear from being irregular.

(iii) Stain should not be too dark or too pale

(iv) There should not be any stain precipitate present on smear.

RBC - pink & WBC - appropriate stain

(v) There should be proper distribution of cells on smear.

2). Cedar ~~of~~ oil used with oil immersion objective as it has same refractive index as the glass of cover slip so that the object is effectively immersed in it. It also increases the resolution, light travels through air at a greater speed than through glass. Thus so, to increase the effective NA of the objective, oil is used to slow down the speed at which light travels

Experiment 11

Date :

DIFFERENTIAL LEUCOCYTE COUNT

AIM

To find out the percentage of different types of WBCs in a peripheral blood smear.

PRINCIPLE

A peripheral blood smear is prepared and stained using Leishman's stain. It is examined under the oil immersion of the compound microscope. 100 WBCs are counted and the percentage of different types is noted.

REQUIREMENTS

Clean, grease free glass slides, Leishman's stain, distilled water, staining rack, cedar wood oil, spirit, cotton, lancet and microscope.

PROCEDURE

A thin uniform peripheral blood smear is prepared and stained using Leishman's stain. Dry it, put an oil drop on the film and focus under the oil immersion objective identify the different types of WBCs which are differentially stained with the eosin and methylene blue. Draw a square in a paper and divide it into 100 columns. Start counting leucocytes from one end of the smear by moving the slide towards the other end. As and when each WBC is identified enter in the column 'N' for neutrophil, 'E' for eosinophil, 'B' for basophil, 'L' for lymphocyte and 'M' for monocyte. When the other end of the slide is reached, move the slide two fields down and then towards the other end. Like this the smear is moved in a zigzag manner till 100 cells are counted and entered. Express the count as percentage.

Identification of different leucocytes

Neutrophil : 10 – 15 micron size

Multilobed nucleus, usually 2-3 lobes connected by chromatin threads, purplish blue in colour. Cytoplasm plenty, bluish pink, with plenty of fine granules, granules are faint pink or lilac in colour, cell outline is usually distinct.

Eosinophil : 10 -15 micron size

Nucleus 2-3 lobed, usually bilobed and spectacle shaped or horseshoe shaped purplish blue in colour. Cytoplasm plenty, pink in color, orange red or brickred coarse granules (strongly acidophilic) which are not plenty, cell outline not distinct.

Basophil : 10 -14 micron size

Nucleus – lobulation incomplete, purplish blue in colour. Cytoplasm plenty, bluish black coarse granules, may overlie the nucleus so that the nucleus may not be clearly seen.

Lymphocyte : Pale blue non-granular cytoplasm and deep purplish blue non-lobulated nucleus. Cell out line is distinct.

Small lymphocyte : 7 – 10 micron size

Nucleus round or slightly indented, fills almost entire cell, cytoplasm scanty, may be seen as a distinct perinulear ring.

Large lymphocyte : 10 – 15 micron size

Nucleus usually indented, does not occupy entire cell. Cytoplasm to nucleus ratio more than small lymphocyte.

Monocyte: 15-22 Microns Size

Nucleus : large, round or oval, deeply indented to have kidney shape, pale purplish blue in colour, usually eccentric. Contains fine rectified chromatin (unlike large lymphocyte)

Cytoplasm : grayish blue (slate grey), plenty, frosted glass appearance.

Variation in number of different cells

Neutrophils : 50-70%

Neutrophilia

Increase in neutrophil percentage above normal. Acute bacterial infections (eg. Tonsillitis, appendicitis, pneumonia, lung abscess, etc.) burns, acute hemorrhage, myocardial infarction, rheumatic fever, primary polycythemia, uremia, gout, diabetic ketoacidosis, chronic myeloid leukemia (CML), poisoning with digitalis, lead, mercury, etc.

Physiological conditions like muscular exercise, meals, newborns, labour, emotional stress, menstruation, pregnancy.

Neutropenia

Decrease in neutrophil percentage below normal. Typhoid, paratyphoid fever, typhus fever, malaria, aplastic anemia, bone marrow irradiation, agranulocytosis, hypersplenism, some viral infections (influenza, rubella, measles, viral hepatitis), some protozoan infections (malaria, kala azar), some drugs (chloramphenicol, phenylbutazone, carbamazepine)

Eosinophils : 1-4%

Eosinophilia

1. Allergic conditions like bronchial asthma, urticaria, hayfever, serum sickness, eczema
2. Parasitic infections like round worm, hook worm, filariasis
3. Tropical eosinophilia (Loeffler's syndrome)
4. Some drugs Eg. Penicillin, iodides, aspirin.

Eosinopenia : ACTH or steroid treatment, Cushing's syndrome, stressful conditions, polycythemia

Basophilia : Small pox, primary polycythemia, chicken pox, measles, myxedema, CML, after splenectomy

Basopenia : Cushing's syndrome, ACTH or steroid therapy

Lymphocytes : 20 -40 %

Lymphocytosis : Increase in lymphocyte percentage

1. Chronic infections like tuberculosis
2. Viral infections
3. A few acute infections like whooping cough (pertussis), infectious mononucleosis (IMN)
4. Lymphatic leukemia
5. Physiological, in infancy and childhood